

## Cytogenetic studies of seven species of Charaxinae (Nymphalidae, Lepidoptera) from Brazil and Peru

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**Abstract** The meiotic chromosomes of seven charaxine species belonging to the genera *Archaeoprepona*, *Prepona* and *Agrias* from Brazil and Peru were investigated with primary and secondary spermatocytes. The haploid numbers of chromosomes were found to be  $n=16$  for *Archaeoprepona demophon* and *Ar. demophoon*,  $n=15$  for *Ar. licomedes*,  $n=9$  for *Ar. am- phimachus* and *Ar. meander*,  $n=12$  for *Prepona pheridamas* and *Agrias claudina*. All these Neotropical butterflies contained one to ten large-sized chromosomes in their metaphase plates. In view of the correlation between chromosome size and modal number of chromosomes ( $n=31$ ) in Nymphalidae, the large-sized chromosomes noticed here might have been formed through chromosome fusion, which resulted in decrease of chromosome number.

**Key words** Charaxinae, *Archaeoprepona*, *Prepona*, *Agrias*, meiotic chromosomes.

### Introduction

To date the chromosomes of nymphalid butterflies have been studied in more than 280 species (Stevens, 1906; Kernewitz, 1915; Beliajeff, 1930; Federlay, 1938; Lorcovič, 1941, 1958; Lesse, 1952, 1953, 1960, 1967*a*, *b*, 1970*a*, *b*; Maeki, 1953*a*, *b*; Maeki & Makino, 1953; Maeki & Remington, 1961; Maeki *et al.*, 1965; Saitoh & Abe, 1969, 1981; Brown *et al.*, 1992; Abe, 1997). Almost half of these species have the haploid number of  $n=31$  which is regarded as a modal number of chromosomes in this butterfly group. The lowest and highest haploid numbers are  $n=12$  in *Brenthis daphne iwatensis* (Saitoh *et al.*, 1985) and  $n=37$  in *Argynnis anadyomene* (Maeki, 1953*a*, *b*; Maeki & Makino, 1953), respectively, in Heliconiinae, Nymphalinae, Limenitidinae, Apaturinae and Charaxinae.

Recently Lesse (1967*a*, *b*, 1970*a*, *b*) presented the chromosome numbers of about 160 species of S. American butterflies, in which *Archaeoprepona demophon* (as *Prepona demophon*) was reported as  $n=16$  in the haploid number. This is markedly lower than the modal number normally counted in Nymphalinae, Heliconiinae, Limenitidinae, Apaturinae and Charaxinae, but no description of either the karyotypes or photographs was given.

From 1987 to 1996 the authors collected seven species of Charaxinae belonging to the genera *Archaeoprepona*, *Prepona* and *Agrias* for chromosome study in the Neotropical region, Brazil and Peru. These genera are systematically closely related to one another, forming a part of the tribe Preponini, and *Archaeoprepona* is known from about 9 species, *Prepona* from about 21 species and *Agrias* from about 7 species (D'Abrera, 1987). In five species of *Archaeoprepona*, one species of *Prepona* and one species of *Agrias*, which the authors examined, all were confirmed as having the haploid numbers less than  $n=16$ , including  $n=9$ , as shown by Lesse (1970*a*) for *Archaeoprepona demophon*. The haploid number  $n=9$  is a new finding being the lowest so far reported. In this report, the authors discuss a plausible mechanism of

decrease in chromosome number in some charaxine species, paying attention to the existence of large-sized chromosomes which have not been found in the nymphalid butterflies carrying  $n=31$ .

## Materials and methods

Seven charaxine species, in total fifteen male specimens, were collected at four localities of Brazil and Peru from 1987 to 1996 (Table 1). The testes taken from live butterfly specimens were fixed in PFA3 fixative. Chromosome preparations were made according to the paraffin sectioning method (10  $\mu\text{m}$ ) and stained with Heidenhein iron-haematoxylin. In the authors' preparations, spermatogonial metaphases were not suitable for chromosome observation due to the cohesion of chromosomes, while many well-spread metaphases could be obtained in the primary (MI) and secondary (MII) spermatocytes. Such metaphase plates were basically composed of dot-like small chromosomes and rather large round chromosomes. So, the number and size of chromosomes were the main subject of this study.

In the paraffin sectioning method, chromosome size occasionally depends on the sectioning angle and sectioning phase for chromosomes. So, the front and rear sections of a given metaphase plate were always checked. In case of difficulty for determination of chromosome size, the number of chromosomes of given size was expressed with a certain range like "3 to 4".

## Results

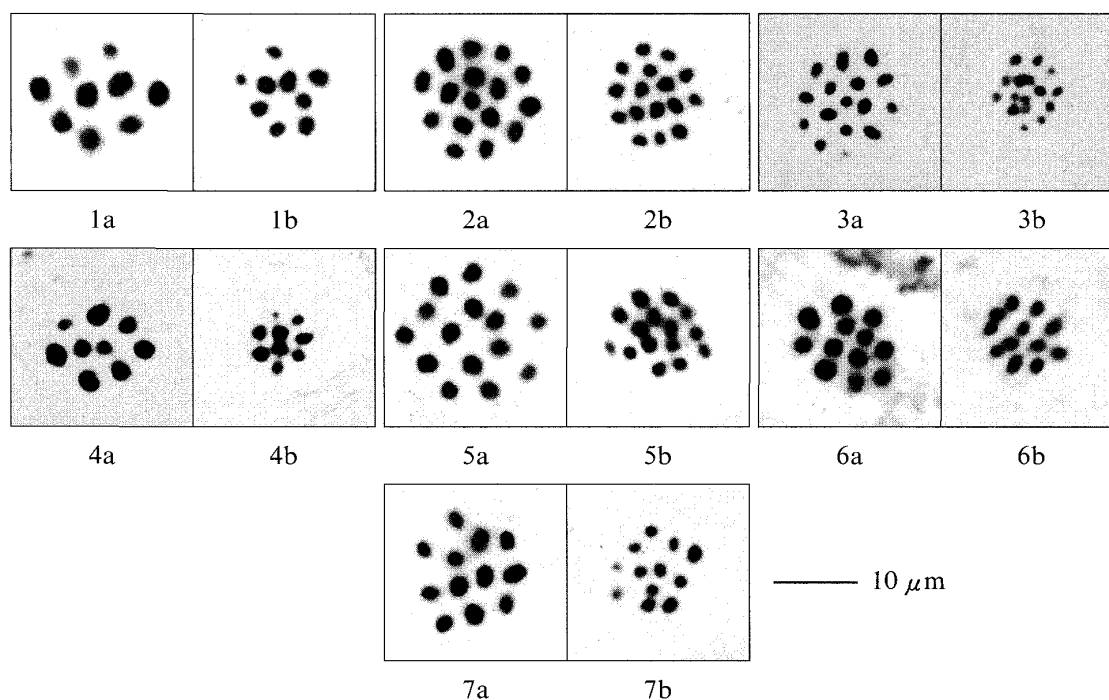
The results of chromosome analysis in MI and MII spermatocytes from the seven charaxine species are summarized in Table 2. No heteroploidy was found in the present study. The haploid numbers of these butterfly species ranged from  $n=9$  to  $n=16$ . In each species, chromosomes were morphologically classified into four types, namely markedly large-sized (LL), large to medium (L-M), and small-sized (S) and markedly small (SS) chromosomes, though this classification was occasionally not clear-cut. In general, the chromosome size of each class was almost two times larger in MI than in MII, apparently reflecting meiotic division. The chromosome findings in each species are described as follows.

### *Archaeoprepona amphimachus* (Fabricius)

The haploid number of this species is  $n=9$ , as determined with only one specimen. This

Table 1. Species of Neotropical charaxine butterflies examined in this study.

species	no. of specimens	collecting date	collecting locality
<i>Archaeoprepona amphimachus</i>	1♂	29. Sept. 1992	Nova orinda (Brazil)
<i>Ar. demophon</i>	2♂	28. Sept. 1992	Nova orinda (Brazil)
	2♂	29. Sept. 1992	Nova orinda (Brazil)
	2♂	1. Jan. 1993	Kanuma (Brazil)
	1♂	2. Jan. 1993	Kanuma (Brazil)
	1♂	3. Jan. 1996	Katumba (Brazil)
<i>Ar. demophoon</i>	1♂	11. Jan. 1987	Tingomaria (Peru)
<i>Ar. meander</i>	1♂	7. Jan. 1987	Tingomaria (Peru)
<i>Ar. licomedas</i>	2♂	28. Sept. 1992	Nova orinda (Brazil)
<i>Prepona pheridamas</i>	1♂	29. Sept. 1992	Nova orinda (Brazil)
<i>Agrias claudina</i>	1♂	28. Sept. 1992	Nova orinda (Brazil)



Figs 1-7. Chromosomes of seven Preponini species from Brazil and Peru. 1. *Archaeoprepona amphimachus* (a: MI, b: MII, Brazil). 2. *A. demophon* (a: MI, b: MII, Brazil; c: MI, Peru). 3. *A. demophoon* (a: MI, b: MII, Brazil). 4. *A. meander* (a: MI, b: MII, Brazil). 5. *A. licomedes* (a: MI, b: MII, Brazil). 6. *Prepona pheridamas* (a: MI, b: MII, Brazil). 7. *Agrias claudina* (a: MI, b: MII, Brazil).

species has 4 LL, 4 L-M, and 1 S chromosomes (Figs 1a and 1b). Both the haploid number and chromosome constitution are quite stable, so far as 10 MI and 32 MII spermatocytes observed are concerned.

#### *Archaeoprepona demophon* (Linnaeus)

All of the eight specimens examined have  $n=16$  as already reported by Lesse (1970b). But the differentiation of chromosome size is rather indistinct as compared with *Ar. amphimachus* (Figs 2a and 2b), and thus the numbers of L-M and S chromosomes could not be determined as fixed numbers (Table 2).

#### *Archaeoprepona demophoon muson* (Cramer)

This species also has  $n=16$ , resembling *Ar. demophon* in its chromosome constitution. But *Ar. d. muson* is apparently different from *Ar. demophon* in which one of the S chromosomes is markedly small, as confirmed by observing consecutive paraffin sections (Figs 3a and 3b). This unusually small chromosome was termed as "SS" here (Table 2).

#### *Archaeoprepona meander* (Cramer)

This species has the haploid number  $n=9$  characterized by 4-5 LL, 3-4 L-M and 1 S, closely resembling *Ar. amphimachus* in chromosome constitution as well as in haploid number of chromosomes, though *Ar. meander* seemed to have one more LL chromosome than *Ar. amphimachus* (Figs 4a and 4b).

*Archaeoprepona licomedes* (Cramer)

The haploid number of this species is  $n=15$ . Differentiation of chromosome size seemed to be of low degree in this species, as shown in Figs 5a and 5b. But most of the spermatocytes examined contained 12–14 L–M and 1–3 S chromosomes.

*Prepona pheridamas* (Cramer)

The haploid number is  $n=12$ , as determined with only one specimen. In most of the spermatocytes examined, all 12 chromosomes were of M–L size without S chromosomes, independent of deflection of the section angle (Fig. 6a). The MII plate of Fig 6b contains mostly M chromosomes due to subtle deflection of the section angle.

*Agrias claudina sardanapalus* (Bates)

This species has  $n=12$  equal to *P. pheridamas* in haploid number, carrying 9–11 L–M and 1–3 S chromosomes (Figs 7a and 7b).

## Discussion

Literature reported to date proved that the modal haploid number of chromosomes in Nymphalinae, Heliconiinae, Limenitidinae, Apaturinae and Charaxinae is  $n=31$ . The haploid numbers found in the present Neotropical species are any of  $n=16$ ,  $n=15$ ,  $n=12$  or  $n=9$ , being apparently far lower than the modal number  $n=31$ . Assuming that the ancestral group of Nymphalidae had the modal number  $n=31$ , the present Neotropical species would be derivative. In other words, these Neotropical species might have evolved in the direction of diminishing chromosomes by means of chromosome fusion or chromosome loss. As an example of the former case, Maeki (1961) reported the chromosomal relationship among three apaturine species, *Apatura metis* (as *ilia*) ( $n=31$ ), *Hestina japonica* ( $n=30$ ) and *Sasakia charonda* ( $n=29$ ). The first species contains only small chromosomes,

Table 2. Summary of chromosome analysis in seven nymphaline species from Brazil and Peru.

species	locality	haploid number of chromosomes	no. of spermatocytes observed		remarks
			I	II	
<i>Archaeoprepona amphimachus</i>	Brazil	$n, 9$	10	32	4LL, 4L–M, 1S
<i>Ar. demophon</i>	Brazil	$n, 16$	7	14	13–14L–M, 2–3S
<i>Ar. demophon</i>	Brazil	$n, 16$	20	23	1L, 2–3S
<i>Ar. demophon</i>	Brazil	$n, 16$	39	15	4L, 2–3S
<i>Ar. demophon</i>	Brazil	$n, 16$	27	14	4L, 2–3S
<i>Ar. demophon</i>	Brazil	$n, 16$	54	58	1–3L, 2–3S
<i>Ar. demophon</i>	Brazil	$n, 16$	26	15	1–3L, 2–3S
<i>Ar. demophon</i>	Peru	$n, 16$	27	25	1–3L, 3S
<i>Ar. demophon</i>	Peru	$n, 16$	14	12	13–14L–M, 2–3S
<i>Ar. demophon</i>	Peru	$n, 16$	12	42	11–12L–M, 3–4S, 1SS
<i>Ar. meander</i>	Peru	$n, 9$	110	47	4–5LL, 3–4L–M, 1S
<i>Ar. licomedes</i>	Brazil	$n, 15$	7	2	12–14L–M, 1–3S
<i>Ar. licomedes</i>	Brazil	$n, 15$	12	8	12–14L–M
<i>Prepona pheridamas</i>	Brazil	$n, 12$	47	12	12L–M, 0S
<i>Agrias claudina</i>	Brazil	$n, 12$	7	11	9–11L–M, 1–3S

and the latter two species contain one and two large-sized chromosomes, respectively. Taking these findings into consideration, he speculated that, in the course of differentiation of *H. japonica* and *S. charonda*, fusion of two pairs of chromosomes might have taken part in the formation of one pair of large chromosomes, resulting in reduction of one bivalent in MI. Maeki (1958) also proposed, for polymorphic occurrence of *Pieris rapae* ( $n=25$ ,  $n=26$ ), chromosomal reduction by means of chromosome loss through elimination into cytoplasm. In case of fusion, the resulting fusion-mediated chromosome may inevitably become larger in size. In the present charaxine species, the spermatocytes of each species contain markedly large, large-to-medium, small and markedly small classes of chromosomes (LL, L-M, S and SS) which are morphologically discernible to each other. The LL chromosomes were found in two *Archaeoprepona* species ( $n=9$ ), and an SS was found only in *Ar. demophon*. Of special interest is the general tendency that the L-M chromosomes of these seven species appear to decrease in number and become larger as the haploid number decreases. These findings may suggest participation of chromosome fusion as in the cases reported by Maeki (1961), especially involvement of more than two bivalents in chromosome fusion in two *Archaeoprepona* species ( $n=9$ ). Apart from the propriety of this notion, *Ar. amphimachus* and *Ar. meander* are karyosystematically close to each other.

Of seven species examined, *Ar. demophon* and *Ar. demophon* have the largest haploid number  $n=16$ . In both species, differentiation of chromosome size seemed to be not so remarkable as compared with the other species with lower haploid number, and reflecting such ambiguity of chromosome size, L-M and S chromosomes varied in number from cell to cell as well as from individual to individual (Table 2). But the latter species has one markedly small chromosome (1SS) which is the only chromosomal marker distinguishing *Ar. demophon* from *Ar. demophon* (Fig. 3a). It is still uncertain whether the SS can be regarded as a marker chromosome specific to this species and stable within species, since only one specimen was examined in this study. Many more specimens should be examined to certify this finding. It may be reasonable to consider, based on the close similarity of chromosomal features, that *Ar. demophon* is systematically closer to *Ar. demophon* than to the remaining three congeners.

Both *Prepona* and *Agrias*, examined in only one specimen each, have an intermediate haploid number ( $n=12$ ), so far as the seven species examined are concerned, showing similar chromosome constitution to each other (Table 2). So, the Neotropical charaxine species, including *Archaeoprepona* species, may be characterized, at least as to the present species, by a low number of chromosomes. The present findings suggest chromosomal evolution of Neotropical charaxine species to a direction of diminution in chromosome number. In order to discover whether this pattern is more general, many other species of Nymphalidae, especially species belonging to *Prepona* and *Agrias*, both of which have well adapted to the tropical jungle zone and differentiated to a large number of species, should be examined focussing on the haploid number and chromosome constitution. This kind of basic chromosome analysis may give a clue to inquiry into speciation process of Neotropical charaxine butterflies.

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## 摘 要

ブラジルおよびペルー産フタオチョウ亜科プレポナ族 7 種の染色体 (阿部 東・熊谷義則)

ブラジルとペルー産のタテハチョウ科フタオチョウ亜科 Preponini に属する 7 種について第 1 精母細胞 (MI) 及び第 2 精母細胞 (MII) の分裂における半数の染色体を調査した。その結果, *Archaeoprepona amphimachus*,  $n, 9$ ; *A. demophon*,  $n, 16$ ; *A. demophoon*,  $n, 16$ ; *A. meander*,  $n, 9$ ; *A. licomedes*,  $n, 15$ ; *Prepona pheridamas*,  $n, 12$ ; *Agrias claudina*,  $n, 12$  であった。これらは、タテハチョウ科における最頻染色体数 (modal chromosome number),  $n, 31$  に較べ少ない染色体数である。これら各種の染色体の中には大型又は大-中型の染色体を含み、この大きな染色体は、染色体の融合によって生じたと考えられた。このことから、Preponini 族では融合によって染色体数を減少させ、進化して来たものと考えられる。

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